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Rinderpest.
Preparation of Anti-Serum

BY

A. W. SHILSTON, M.R.C.V.S.
Assistant Bacteriologist in charge of the Office of Imperial Bacteriologist, Muktesar Laboratory.

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Rinderpest.

Preparation of Anti-serum.[1]

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DURING the past ten years the procedures adopted in the preparation of anti-rinderpest serum at the Muktesar laboratory have been frequently revised and improved as the result of experiments directed to finding a means of raising the potency of the serum and effecting economies in its manufacture. The latter consideration became more urgent as the demand for serum increased owing to the supply of susceptible hill cattle, employed in its production, being strictly limited ; the introduction by Holmes [2] of plains buffaloes for serum making greatly lessened this difficulty but hill cattle were still necessary for maintaining and furnishing the rinderpest virus for hyperimmunization and as test animals in the standardization of the serum.

In 1914-15 the issues of anti-rinderpest serum rose to 13,88,560 doses at 5 c.c. per dose, and the demand for anthrax and hæmorrhagic septicæmia sera also greatly increased. Difficulties of accommodation and fodder supply for the cattle now made it necessary to ascertain whether, in view of a possible further rise in the demand, any modifications of the existing methods could be effected with the object of increasing the yield of serum proportionately to the number of cattle employed.

A comparative study of the yields resulting from different methods of separating the serum from the blood was undertaken by Dr. Norris,[3] Physiological Chemist of the Muktesar laboratory, and as a result of his investigation, a combined centrifuging and clotting method has been introduced in place of the former practice of defibrinating the blood before centrifugalization.

Recent observations in the preparation of anti-diphtheritic, antitetanic and other sera have shown that antitoxin content reaches its maximum from five to seven days after injection of the antigen (toxin, culture, etc.) into the serum-making animal and that from this time the potency of the serum falls fairly rapidly.

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[1] Results arrived at in October 1915.

[2] Holmes, J. D. E., Rinderpest. *Indian Civil Veterinary Department Memoir* No. 3, 1911, p. 98.

[3] Norris, R. V. A comparison of the "Defibrination" and "Oxalate" methods of serum preparation, *Bulletin No. 60, Agri. Research Institute, Pusa.*

Little attention appears to have been given to this point in the case of anti-rinderpest serum but since a reduction of the interval between injection and bleeding would effect a saving both in the cost of feeding and in the number of animals required for serum making, the matter is one of some importance when a large output of serum has to be maintained.

The early workers on anti-rinderpest serum allowed a considerable interval to elapse between the last injection of virulent blood and the first bleeding for serum but there is a curious absence of precise information on this point in their reports ; the bleedings were usually continued at intervals of from seven to ten days for three or more bleedings.

Kolle and Turner [1] in their report to the Secretary for Agriculture, South Africa, give no details of the actual bleedings when describing the method of preparation of the serum, nor does Turner [2] furnish particulars on this point. Rogers,[3] who states that he followed the method of Kolle and Turner, merely remarks that bleedings were made once a week for three weeks.

Lingard [4] in describing his rapid method of preparing serum states that the first bleeding was taken 14 days after the termination of the reaction following injection; and adds "If the reaction lasted 8 days, bleeding would be performed for the first time on the 22nd day after the injection of virulent blood." This method of allowing an interval of three weeks after injection and then bleeding weekly, was followed at the Muktesar laboratory up to the year 1911 when Holmes [5] showed that a more potent serum was obtained by bleeding at 14 to 16 days after injection of the virulent blood and giving a second bleeding two days later ; he adopted this procedure of two bleedings after each injection as the routine method for serum manufacture and it has been in operation up to the present time.

At the serum laboratory of the Bureau of Agriculture, Philippine Islands, Jobling [6] adopted the method then practised at Muktesar; he states: "Following Roger's plan the animals after receiving 1000 c.c. of virulent blood are allowed to remain until the temperature becomes normal. They are then bled three times with an interval of one week between each bleeding." But the length of time considered necessary before taking the first bleeding is not stated.

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[1] Kolle and Turner. Report on progress of Research Work at Kimberley, *Veterinary Journal*, Vol. XLV, 1897, p. 462.

[2] Turner, G. Rinderpest in South Africa, *The Journal of Tropical Veterinary Science*, Vol. 1, 1906, p. 269.

[3] Rogers, L. Report on Rinderpest, 1900, p. 89.

[4] Lingard, A. Report on Rinderpest, 1905, p. 13.

[5] Holmes, J. D. E. *Loc. cit.*

[6] Jobling, J. W. Preliminary Report on the Study of Rinderpest, *Bulletin No. 4, Bureau of Government Laboratories, Manila, Philippine Islands*, 1903, p. 17.

Later reports from the same laboratory by Ruediger [1] and Ward and Wood [2] describe the method of serum preparation that is followed and give seven to ten days as the interval allowed between injection and the first bleeding, two further bleedings being taken at intervals of one week.

No reference has been found to any experiments designed to ascertain the potency of serum taken at varying intervals after inoculation of virulent rinderpest blood.

Holmes [3] tested the value of serum from first, second and third bleedings from plains bulls, hill bulls and buffaloes. Three animals were used in each case for the production of the serum and the bleedings were taken on the 15th, 18th and 23rd days after injection but as the sera from the different bleedings were not tested at uniform doses it is difficult to make any satisfactory comparisons from the results.

The series of observations described in this paper were carried out to ascertain the potency of sera taken 8, 12 and 16 days after the injection of virus and to compare the results with those given by sera taken 15 and 17 days after injection ; the latter intervals were those allowed in the routine method of serum manufacture followed at the Muktesar laboratory, the bleedings being taken at the rate of 6 c.c. and 8 c.c. per lb. body weight respectively. The three bleedings at four days intervals were all taken at the rate of 6 c.c. per lb.

Previous experience had shown that cattle would stand bleeding to this extent with little or no interference to their health and it seemed probable that the potency of the sera from the second and third bleedings would not be found to diminish as rapidly as when intervals of a week are allowed between the bleedings ; the saving in time would also be a consideration. Tests were made of the potency of the mixed sera from the two series and were repeated on a large scale using both hill bulls and buffaloes for serum production.

As the main purpose of the experiments was to provide an increased yield of serum which should be at least of equal potency to that prepared by the existing routine method, records were kept of the amounts of serum obtained at each bleeding and the total yields given under the two systems were compared.

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[1] Reudiger, E. H. Observations on Cattle Plague, *The Philippine Journal of Science,* Vol. IV, s. B., No. 5, 1909, p. 384.

[2] Ward, A. R. and Wood, F. W. Experiments on the Efficiency of Anti-Rinderpest Serum, *Bulletin No. 19, Bureau of Agriculture, Manila, Philippine Islands,* 1912, p. 19.

[3] Holmes, J. D. E. Rinderpest. *Memoirs of the Department of Agriculture in India. Veterinary Series,* Vol. II, No. 2, Nov. 1913, p. 55.

Throughout the experiments the potency of the sera was tested on susceptible hill bulls, six for each test, by injecting them simultaneously with virulent rinderpest blood and graduated quantities of the serum.

In the routine serum testing, doses of 36 c.c., 72 c.c. and 108 c.c. per 600 lb. body weight, two bulls at each dose, are employed, these amounts being equivalent to 2 c.c., 4 c.c. and 6 c.c., respectively in the case of plains animals [1]; but in order to gain a more accurate estimate of the relative values of the sera, the doses throughout the experiments were fixed at 27 c.c., 54 c.c. and 81 c.c. per 600 lb. body weight, equal to 1½ c.c., 3 c.c. and 4½ c.c. respectively for plains cattle.

At each test two hill bulls were inoculated with virulent blood alone to serve as controls; after developing symptoms of acute rinderpest these were bled to death to provide virulent blood for hyperimmunization.

EXPERIMENT I.

To compare the values of sera taken 8, 12 and 16 days after the injection of virulent rinderpest blood with those of sera taken 15 and 17 days after a similar injection.

Ten hyperimmune serum-making hill bulls were selected and divided into two series, A and B, so that the five animals of each series had received an approximately equal number of previous injections and bleedings.

All were injected on the same day with a mixture of virulent rinderpest blood and potassium citrate solution, the doses, which varied from 8 to 10 c.c. of the mixture per lb. body weight, being regulated according to the number of previous injections the animals had received.

The animals of series A were bled 8, 12 and 16 days later at the rate of 6 c.c. per lb. body weight and the sera labelled A 1, A 2 and A 3 respectively.

The animals of series B were bled on the 15th day at the rate of 6 c.c. and on the 17th day at the rate of 8 c.c. per lb. body weight; the sera being labelled B 1 and B 2 respectively.

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[1] For convenience of issue the standardized dose of serum for plains cattle is taken at 5 c.c. per 600 lb. body weight though owing to the varying susceptibility possessed by different animals this only applies to the least susceptible; in other cases more than a single dose must be employed. Owing to their uniform high susceptibility hill bulls are most suitable for test purposes and as observations have shown that they require eighteen times the dose of serum necessary for plains animals of low susceptibility, the test doses are fixed accordingly; only serum which protects hill bulls at 72 c.c. or less per 600 lb. body weight (equal to 4 c.c. for plains cattle) is issued.

Serum-making Bulls.

SUMMARY.

SERIES A.—*Three bleedings at 6 c.c. per lb. body weight.*

Hill Bull No.	Virulent blood and potassium citrate injected c.c.	Bled on 8th day c.c.	Total Serum A 1. c.c.	Bled on 12th day c.c.	Total Serum A 2. c.c.	Bled on 16th day c.c.	Total Serum A 3. c.c.
3,385	2,400	1,900	4,250	1,900	4,950	1,900	5,500
4,047	2,000	1,600		1,700		1,700	
3,925	2,000	2,100		1,700		1,500	
3,279	2,600	2,000		2,000		2,000	
4,024	1,600	1,300		1,500		1,400	
TOTAL	10,600	8,900	4,250	8,800	4,950	8,500	5,500

SERIES B.—*Two bleedings at 6 c.c. and 8 c.c. per lb. body weight respectively.*

Hill Bull No.	Virulent blood and potassium citrate injected c.c.	Bled on 15th day c.c.	Total Serum B 1. c.c.	Bled on 17th day c.c.	Total serum B 2. c.c.
4,674	1,200	900	3,250	1,400	4,500
3,851	2,200	1,800		2,500	
4,473	2,200	1,600		2,100	
4,493	1,800	1,500		2,100	
4,311	1,800	1,400		1,900	
TOTAL	9,200	7,200	3,250	10,000	4,500

Tests of Sera A1, A2, A3, B1 and B2.

SUMMARY.

SERUM A1.—*Test animals and controls inoculated with 0·5 c.c. virulent blood from hill bull No. 5333—temperature 40° C., vesicles 6th day of attack.*

No. of test animal, Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature reaction	Vesicles	Result
5,357	3	194	27	8·8	40·5	10th day	Moderate reaction. Recovery.
5,355	3	210	27	9·5	39·6	nil	Slight reaction. Recovery.
5,358	1½	186	54	16·8	40·2	8th day	Moderate reaction. Recovery.
5,356	4	216	54	19·5	40·3	11th day	Moderate reaction. Recovery.
5,359	3	172	81	23·2	39·0	nil	No reaction. Recovery.
5,354	3½	226	81	30·5	40·1	nil	Very slight reaction. Recovery.

Controls.

Hill Bull 5360. Severe attack, temperature 39·6°C. Vesicles 8th day. } Bled to death
Hill Bull 5361. Severe attack, temperature 40·6°C. Vesicles 8th day. } 9th day.

SERUM A2.—*Test animals and controls inoculated with 0·5 c.c. virulent blood from hill bull 5718, temperature 40·3°C., vesicles 6th day.*

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actuai Dose c.c.	Maximum temperature	Vesicles	Result
5,747	3	176	27	7·9	40	nil	Moderate reaction. Recovery.
5,749	1½	176	27	7·9	40·6	,,	Moderate reaction. Recovery.
5,750	2	180	54	16·2	39·3	,,	Slight reaction. Recovery.
5,746	2	190	54	17·1	40·5	,,	Moderate reaction. Recovery.
5,377	3	188	81	25·4	40·5	,,	Slight reaction. Recovery.
5,372	2	200	81	27	39·7	,,	Slight reaction. Recovery.

Controls.

Hill Bull 5756. Very severe attack, temperature 41°C. Vesicles 7th day. } Bled to death
Hill Bull 5755. Very severe attack, temperature 40·9°C. Vesicles 6th day. } 8th day.

SERUM A3.—*Tested with serum A2. Virulent blood from the same hill bull being employed.*

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,748	2½	172	27	7·7	40·3	nil	Moderate reaction. Recovery.
5,596	3	174	27	7·8	40·4	8th day	Died 13th day.
5,752	2	180	54	16·2	40·7	nil	Moderate reaction. Recovery.
5,751	3	196	54	17·6	40·4	,,	Moderate reaction. Recovery.
5,378	3	180	81	24·3	39·5	,,	Very slight reaction. Recovery.
5,380	2	192	81	25·9	40·7	,,	Slight reaction. Recovery.

SERUM B1.—*Test animals and controls inoculated with 0·5 cc. virulent blood from hill bull 5432, temperature 40°C., vesicles 6th day of attack.*

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,480	2	170	27	7·6	41	nil	Moderate reaction. Recovery.
5,482	2	186	27	8·37	40·5	,,	Moderate reaction. Recovery.
5,481	4	210	54	18·9	39·8	,,	Slight reaction. Recovery.
5,479	6	298	54	26·8	39·8	,,	Slight reaction. Recovery.
5,478	5	406	81	54·8	40·5	,,	Slight reaction. Recovery.
5,477	12	412	81	55·6	40·7	,,	Slight reaction. Recovery.

Controls.

Hill Bull 5474. Severe reaction 40·1°C. Vesicles 6th day. Bled out 8th day.
Hill Bull 5475. Severe reaction 40·5°C. Vesicles 8th day. Bled out 9th day.

SERUM B2.—*The same virulent blood and controls as for Serum B1.*

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,488	3	174	27	7·8	40·5	nil.	Moderate reaction. Recovery.
5,486	3	180	27	8·1	40·7	8th day	Died 12th day.
5,487	4	200	54	18	40·0	10th day	Moderate reaction. Recovery.
5,485	7	240	54	21·6	40·0	nil	Slight reaction. Recovery.
5,484	12	318	81	42·9	39·5	,,	Slight reaction. Recovery.
5,483	10	378	81	51	40·2	,,	Slight reaction. Recovery.

CONCLUSIONS.

A comparison of the results of the above five tests shows that—

(1) the rinderpest antibodies were present in the serum of hyperimmune animals in full amount 8 days after the injection of virulent blood,

(2) at the second bleeding on the 12th day the antibodies were still present in sufficient quantity for 27 c.c. per 600 lb. body weight to protect hill bulls against the injection of virulent blood, which is equal to 1·5 c.c. per 600 lb. in the case of plains animals,

(3) the serum from a third bleeding four days later was still protective in doses of 54 c.c. for hill bulls or 3 c.c. for plains bulls per 600 lb. body weight,

(4) the serum from a first bleeding 15 days after the injection of virulent blood was fully protective in doses of 27 c.c. for hill bulls or 1·5 c.c. for plains bulls, but at a second bleeding on the 17th day the potency had fallen so that 54 c.c. per 600 lb. body weight were required to give full protection to hill bulls.

EXPERIMENT II.

To compare the value of the mixed serum obtained from three bleedings, 8th, 12th and 16th days after injection with that of serum from two bleedings taken on the 15th and 17th days.

The three sera A1, A2 and A3 of series A and the two sera B1 and B2 of series B were mixed separately in the proportions of the total yields from each bleeding and labelled A123 and B12 respectively; these sera were then tested at the same time.

Tests of Sera A123 and B12.

SUMMARY.

SERUM A123.—*Both sets of test animals and the controls inoculated with 0·5 c.c. virulent blood from hill bull No. 5519 temperature 40·6°C., vesicles 6th day.*

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,577	2½	146	27	6·5	39·6	nil	Slight reaction. Recovery.
5,575	3	162	27	7·2	40·8	10th day	Moderate reaction. Recovery.
5,576	2	168	54	15·1	39·8	nil	Slight reaction. Recovery.
5,573	3	181	54	16·2	40·8	,,	Moderate reaction. Recovery.
5,574	1½	204	81	27·5	39·9	,,	Very slight reaction. Recovery.
5,578	4½	224	81	30·2	39·8	,,	Very slight reaction. Recovery.

SERUM B12.

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,571	1½	168	27	7·5	40·8	8th day	Moderate reaction. Recovery.
5,567	2	178	27	8	40·1	nil	Moderate reaction. Recovery.
5,568	6	179	54	16·1	39·2	„	Slight reaction. Recovery.
5,569	1½	183	54	16·4	40·6	„	Slight reaction. Recovery.
5,572	2	194	81	26·1	40·6	„	Moderate reaction. Recovery.
5,570	2	252	81	34	40·2	10th day	Moderate reaction. Recovery.

Controls.

Hill Bull 5565. Severe attack, temperature 40·7°C. Vesicles 7th day. Bled to death. 8th day.

Hill Bull 5621. Severe attack, temperature 40·2°C. Vesicles 6th day. Bled to death. 7th day.

CONCLUSIONS.

The above results show that the mixed serum from 3 bleedings on the 8th, 12th and 16th days after injection and that from 2 bleedings on the 15th and 17th days were protective in doses of 27 c.c. for hill bulls, or 1·5 c.c. for plains bulls, per 600 lb. body weight, but the " 3 bleedings " serum gave a distinctly better test.

EXPERIMENT III.

To compare the value of the mixed serum from a second series of 3 bleedings on the 8th, 12th and 16th days after the injection of virulent blood in animals previously bled three times, with the mixed serum from 2 bleedings on the 15th and 17th days in animals previously bled twice.

The advantage of injecting the rinderpest virus into an animal when in a negative phase and the detrimental effect on the potency of the serum of doing so when the animal's blood already contains a considerable quantity of antibody, were demonstrated by Holmes.[1]

Animals bled three times at the rate of 6 c.c. per 600 lb. body weight may be presumed to lose a greater amount of the rinderpest antibodies than those bled twice at the rate of 6 c.c. and 8 c.c. respectively, so that the response in antibody formation to the injection of a given quantity of antigen (virulent blood) should therefore be greater in the former than in the latter and a more potent serum be produced. The object of the present experiment was to determine whether this could be demonstrated.

The same ten hill bulls used in series A and B received an injection of virulent blood and potassium citrate solution 12 days after the last bleeding in the previous experiments. The bulls of series A were bled on the 8th, 12th and 16th days after injection at the rate of 6 c.c. per lb. body weight and the total serum obtained from the three bleedings mixed; Serum A456. The bulls of series B were bled on the 15th and 17th days after injection as before and the total serum mixed; Serum B34.

Serum-Making Bulls.

SUMMARY.

SERIES A.—*Second set of three bleedings at 6 c.c. per lb. body weight.*

Hill Bull No.	Virulent blood and potassium citrate injected c.c.	Bled on 8th day c.c.	Total Serum A4. c.c.	Bled on 12th day c.c.	Total Serum A5. c.c.	Bled on 16th day c.c.	Total Serum A6 c.c.
3,385	2,400	1,800	3,500	1,800	4,600	1,800	4,850
4,047	2,000	1,600		1,600		1,600	
3,925	2,000	1,600		1,500		1,500	
3,279	2,600	2,000		1,900		1,900	
4,024	1,600	1,300		1,300		1,300	
TOTAL .	10,600	8,300	3,500	8,100	4,600	8,100	4,850

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[1] Holmes, J. D. E. Rinderpest. *Indian Civil Veterinary Department Memoir*, No. 3, 1911.

SERIES B.—*Second set of two bleedings at 6 c.c. and 8 c.c. per lb. respectively.*

Hill Bull No.	Virulent blood and potassium citrate injected c.c.	Bled on 15th day c.c.	Total Serum B 3. c.c.	Bled on 17th day c.c.	Total Serum B 4. c.c.
4,674	1,200	1,000	3,650	1,400	5,650
3,851	2,200	1,800		2,500	
4,473	2,200	1,600		2,200	
4,493	1,800	1,400		1,900	
4,311	1,800	1,300		2,000	
TOTAL .	9,200	7,100	3,650	10,000	5,650

Tests of Sera A456 and B34.

SUMMARY.

SERUM A456.—*The test animals in both cases and the controls were inoculated with 0·5 c.c. virulent blood from hill bull 5600, temperature 40·5° C. vesicles on 6th day.*

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,738	3	176	27	7·9	40·5	8th day	Died 14th day.
5,736	5	188	27	8·4	40·5	nil	Moderate reaction Recovery.
5,735	3	196	54	17·6	39·9	,,	Slight reaction. Recovery.
5,733	3	212	54	19	40·8	9th day	Moderate reaction. Recovery.
5,729	3	216	81	29·1	40·6	10th day	Moderate reaction. Recovery.
5,728	5	280	81	32·4	40·7	8th day	Moderate reaction. Recovery.

SERUM B34.

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,734	3	188	27	8·4	41·4	8th day	Died 11th day.
5,598	3	192	27	8·6	40·2	nil	Moderate reaction. Recovery.
5,737	3	198	54	17·8	40·8	8th day	Died 18th day.
5,730	4	206	54	18·5	39·8	nil	Moderate reaction. Recovery.
5,732	7	224	81	30·2	39·9	nil	Moderate reaction. Recovery.
5,731	2	240	81	32·4	41·0	10th day	Moderate reaction. Recovery.

Controls.

Hill Bull 5727. Acute attack, temperature 39·9°C. Vesicles 6th day. Bled out 7th day.
Hill Bull 5591. Severe attack, temperature 40·2°C. Vesicles 6th day. Bled out 6th day.

CONCLUSIONS.

Neither of the mixed sera A456 and B34 gave as good tests as the sera A123 and B12, possibly on account of greater susceptibility of the batch of hill bulls used for the tests or of greater virulence of the virus, but the three-bleedings mixed serum again proved slightly better than the two-bleedings mixed serum. The difference was however not sufficiently marked to draw any definite conclusion as to a greater production of antibodies having occurred in the animals previously bled three times than in those only bled twice.

EXPERIMENT IV.

In consequence of the foregoing results it was decided to compare the 3 bleedings and 2 bleedings systems on a large scale in the routine manufacture of anti-rinderpest serum, testing the two methods in hill bulls and buffaloes separately.

Accordingly without making any selection a proportion of the serum-making hill bulls and buffaloes were bled three times, on the 8th, 12th and 16th days after injection while the remainder were bled as previously, that is on the 15th and 17th days after injection of virus.

In this way 64 hill bulls were given three bleedings which yielded in all 162,580 c.c. of mixed serum ; Hill bull 3 bleedings serum.

One hundred and three hill bulls were bled twice and gave 220,000 c.c. of mixed serum ; Hill bull 2 bleedings serum.

Eighteen buffaloes were bled three times and gave 63,525 c.c. of mixed serum ; Buffalo 3 bleedings serum ; and 58 buffaloes bled twice gave 156,600 c.c. of mixed serum ; Buffalo 2 bleedings serum.

Each serum was then tested on 4 hill bulls—two at 27 c.c. and two at 54 c.c. per 600 lb. body weight ; the largest dose of 81 c.c. per 600 lb. was omitted in this test.

All the test animals and controls were inoculated with 0·5 c.c. of virulent blood from hill bull 5785 taken at the height of a severe attack of rinderpest ; temperature 40·3° C., vesicles 7th day.

Tests of Sera.

SUMMARY.

Serum Hill Bull 2 Bleedings.

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,830	1	174	27	7·83	40	9th day	Moderate reaction. Recovery.
5,831	3	182	27	8·19	39·8	,,	Moderate reaction. Recovery.
5,829	2	212	54	19·08	40·3	nil	Moderate reaction. Recovery.
5,828	1½	227	54	20·43	40·3	,,	Moderate reaction. Recovery.

Serum Hill Bull 3 Bleedings.

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,739	1	176	27	7·92	40·7	nil	Moderate attack. Recovery.
5,738	1½	190	27	8·55	40·6	9th day	Moderate attack. Recovery.
5,837	2	208	54	18·72	39·7	nil	Slight attack. Recovery.
5,836	2	226	54	20·34	40·5	8th day	Moderate attack. Recovery.

Serum Buffalo 2 Bleedings.

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,834	1½	196	27	8·82	40·5	9th day	Moderate attack. Recovery.
5,835	3	206	27	9·27	40·8	,,	Severe attack. Recovery.
5,832	1	197	54	17·73	40·6	nil	Slight attack. Recovery.
5,833	2	226	54	20·34	40·2	,,	Slight attack. Recovery.

Serum Buffalo 3 Bleedings.

No. of test animal Hill Bull	Age yrs.	Weight lb.	Dose per 600 lb. weight c.c.	Actual Dose c.c.	Maximum temperature	Vesicles	Result
5,827	2	192	27	8·64	40·6	nil	Slight attack. Recovery.
5,826	1	194	27	8·73	39·5	,,	Very slight attack. Recovery.
5,825	3	196	54	17·64	40·2	,,	Slight attack. Recovery.
5,824	2	206	54	18·36	40·5	,,	Slight attack. Recovery.

Controls.

Hill Bull 5840. Severe attack, temperature 40·7°C. Vesicles 7th day. Bled out 9th day.
Hill Bull 5842. Severe attack, temperature 40·8°C. Vesicles 7th day. Bled out 9th day.

CONCLUSIONS.

(1) The mixed sera obtained by both the 3 bleedings and 2 bleedings methods from hill bulls and buffaloes were protective in doses of 27 c.c. for hill bulls or 1·5 c.c. for plains animals, per 600 lb. body weight; this is less than a third of the standard dose issued.

(2) The mixed sera obtained from hill bulls by the two methods were of about equal value according to the tests.

(3) The mixed serum obtained from buffaloes by the three-bleedings method was markedly stronger than that obtained by the two-bleedings method and would probably protect in even smaller doses than 1.5 c.c. per 600 lb. body weight in plains animals.

EXPERIMENT V.

To compare the yields of serum by the 3 bleedings and 2 bleedings methods.

The observations were made at the same time as those of Experiments I to III on the 10 hill bulls of Series A and B and so cover six bleedings of the former and four bleedings of the latter series. Records were kept of the body weights of the animals throughout the experiments, the amounts of blood taken and the yields of serum at each bleeding.

The following tables show the total figures for each series of 5 animals :—

Series A.—*6 bleedings (2 sets of 3 bleedings at 6 c.c. per lb. each on 8th, 12th and 16th days after each injection).*

Bleeding	Rate per lb.	Total body weight lb.	Total blood c.c.	Total fibrin c.c.	Total corpuscles c.c.	Total Serum c.c.
1st . . .	6 c.c.	1,470	8,900	1,100	3,550	4,250
2nd . . .	”	1,455	8,800	1,150	2,700	4,950
3rd . . .	”	1,424	8,500	1,000	2,000	5,500
	Reinjected with virus.					
4th . . .	6 c.c.	1,445	8,350	1,850	3,000	3,500
5th . . .	”	1,419	8,100	1,250	2,250	4,600
6th . . .	”	1,406	8,100	1,050	2,200	4,850
	Total .	8,619	50,750	7,400	15,700	27,650

SERIES B.—*4 bleedings (2 sets of 2 bleedings at 6 and 8 c.c. per lb. on 15th and 17th days after each injection).*

Bleeding	Rate per lb.	Total body weight lb.	Total blood c.c.	Total fibrin c.c.	Total corpuscles c c.	Total Serum cc.
1st . . .	6 c.c.	1,263	7,200	1,150	2,800	3,250
2nd . .	8 c.c.	1,212	10,000	1,750	3,750	4,500
		Reinjected	with	virus.		
3rd . . .	6 c.c.	1,279	7,200	1,000	2,550	3,650
4th . . .	8 c.c.	1,267	10,200	1,400	3,150	5,650
	TOTAL .	5,021	34,600	5,300	12,250	17,050

The following summary is obtained from a comparison of the above tables :—

—	Series A (6 bleedings)	Series B (4 bleedings)
Average total weight of hill bulls during test .	1,439 lb.	1,255 lb.
Total blood taken	50,700 c.c.	34,600 c.c.
Total serum obtained	27,650 c.c.	17,050 c.c.
Total blood taken per lb. body weight . .	35·23 c.c.	27·56 c.c.
Total yield of serum per lb. body weight . .	19·21 c.c.	13·58 c.c.
Percentage of serum from blood . . .	54·5 per cent.	49·2 per cent.
Alteration in total body weight during test .	64 lb. loss	4 lb. gain.

A comparison of the figures for the 3 bleedings and 2 bleedings methods in the above observation shows—

(1) an increased yield of serum after each injection of 2·81 c.c. per lb. body weight by the former method (9·6 c.c.) as against the latter (6·79 c.c.) ; this means a total increased yield of 41·4 per cent,

(2) an increased percentage yield of serum from the blood taken of 5·3 per cent. by three bleedings (54·5 per cent.) over that from two bleedings (49·2 per cent.) the proportion of serum increasing with each successive bleeding of a series.

The results of the above experiment were confirmed by the yields of serum obtained in Experiment IV, large series; the figures are given in the following table :—

Animal	Total number bled	No. of bleedings	Total yield of serum c.c.	Average yield per head c.c.
Hill bull	103	2	220,000	2,135
" "	64	3	162,580	2,540
Buffalo	58	2	156,600	2,700
"	18	3	63,525	3,529

In this observation the figures show an increase of serum from hill bulls of 19 per cent. and from buffaloes of 30·7 per cent. by the 3 bleedings method as compared with the 2 bleedings method, but as the weights of the animals were not recorded, this observation has not the same accuracy as the preceding one.

SUMMARY.

1. The interval allowed between the injection of the rinderpest blood and citrate solution mixture and the first bleeding for serum in hyperimmune animals may, with advantage, be reduced to 8 days, as the immune bodies are then present in full amount.

2. By taking three bleedings at the rate of 6 c.c. per lb. body weight on the 8th, 12th and 16th days after injection a mixed serum was obtained of equal (hill bulls) or increased (buffaloes) potency to that obtained by taking two bleedings 15 and 17 days after injection at the rate of 6 c.c. and 8 c.c. per lb. body weight respectively, as was done in the routine method followed at the Muktesar laboratory.

3. The actual yield of serum after each injection was increased from 6·79 c.c. per lb. body weight by the two bleedings system to 9·6 c.c. by the three bleedings system or an additional 2·81 c.c. of serum per lb. body weight; an increase of 41·4 per cent. on the former output.

With an average issue of over 500,000 c.c. of anti-rinderpest serum per month this increase without additional expenditure represents a very large reduction in the cost of manufacture.

MUKTESAR,
June 1st, 1916.

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